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Comparison of CALUX-TEQ values with PCB and PCDD/F measurements in human serum of the Flanders Environmental and Health Study (FLEHS)

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Abstract

In 1999, a campaign of the Flemish Ministry of Health, Belgium was set up to assess pollutant concentrations and related health effect biomarkers in humans living in two regions of Flanders. The study was called the 'Flemish Environment and Health Study' (FLEHS). Concentrations of selected organochiorine pesticides, polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins (PCDD) and flirans (PCDF) were measured by gas chromatography–mass spectrometry in 47 pooled human serum samples originating from 200 individual women between 50 and 65 years living in two Flemish regions. The CALUX® (Chemical-Activated Luciferase gene eXpression) bioassay was assessed on the same pools. The correlation between CALUX-TEQ and total TEQ (sum of PCDD/PCDF, non- and mono-*ortho* PCBs) varied from 0.43 to 0.73 for the rural and urban region, respectively. The mean value for the total TBQ (75 pg WHO-TEQ/g fat) was two times higher than the mean TEQ value determined with the CALUX bioassay (36 pg TEQ/g fat). This shows that the assessment of dioxin-like exposure by these two measurements was different. However, regional differences in concentrations were observed for neither total TEQs, nor CALUX-TEQs. It was concluded that the CALUX® can be an alternative screening tool for biomonitoring purposes, especially when the objective is to compare different groups of people (e.g. living in different regions).

Keywords: CALUX®; Polychiorinated biphenyls; Polychlorinated dibenzodioxins and furans; GC–MS; TEQ; Human serum; Belgium

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1. Introduction

Past measurements of polychlorinated dibenzo-*p*-dioxins (PCDD) and flirans (PCDF) in human milk showed that Belgium had among the highest values in Europe (Tarkowski and Yrjänheikki, 1989). Measurements of PCDD/Fs in Belgian human serum were done only very recently. In Wallonia (Belgium), PCDD/F and heavy metal loads were measured in the general population in 1999. A mean PCDD/F concentration of 36.7 pg 1-TEQ/g fat was found in a group of 54 men and women (mean age 50 years, range 10–80 years) living near to a municipal waste incinerator. This was 34% higher than the mean concentration (27.2 pg 1-TEQ/g fat) measured in 32 persons of similar age range living in a rural area (Bernard, personal communication).

In 1999, a campaign of the Flemish Ministry of Health, Belgium, was set up to assess regional differences in pollutant concentrations and related health effect biomarkers in humans. The study was called the 'Flanders Environmental and Health Study' (FLEHS). This was the most elaborated study up to date done in Belgium. Concentrations of selected organochlorine pesticides, polychiorinated biphenyls (PCB) and PCDD and PCDF were measured by gas chromatography–mass spectrometry (GC–MS) in 47 pooled human serum samples originating from 200 individual women between 50 and 65 years living in two areas of Flanders, Belgium (Koppen et al., 2001). Pooling of serum samples was necessary in order to obtain sufficient material to perform all mentioned analyses. This study is unique since it was one of the few studies where all dioxin-like compounds were measured in the same population. Beside chemical determination of these pollutant concentrations (Koppen et al., 2001), another objective was its comparison with the information obtained by the CALUX® (Chemical-Activated Luciferase gene eXpression) bioassay. This assay needs only low volumes of serum (2–5 ml) and can be used as screening method for analysis of dioxin-like compounds binding to the aryl hydrocarbon receptor (AhR). The comparison between CALUX-TEQ values with the chemical TEQs obtained from PCB and PCDD/F measurements in human serum is discussed here. Details of measurement results and correlation data between groups of polychlorinated aromatic hydrocarbons were presented elsewhere (Koppen et al., 2001; Covaci et al., 2001).

2. Materials and methods

2.1. Study area and population

The rural area (Peer) is situated 15–25 km from the nearest non-ferrous and chemical plants and lies away from motorways. The urban area (two suburbs of Antwerp city) is located 11–13 km SE from the chemical and petrochemical industry established in the harbour of Antwerp. The study group consisted of 200 healthy women between 50 and 65 years old from Antwerp ($n = 100$) and Peer ($n = 100$) recruited randomly between June and September 1999. The initial selection comprised of 2898 randomly selected women between 50 and 65 years old, which were contacted by letter. About half of the 40.1 and 30.8% responders in Antwerp and Peer respectively, were further selected ($N = 685$) because of compliance to the following four criteria: non- or ex-smoker, minimal residence time of 10 years in the study area, working in the town of residence or at home and exclusion ofjobs with specific risks of exposure. From these women, 255 were contacted by telephone, and 200 individuals decided to participate in the study. Bach participant filled in an informed consent and the study was approved by the Ethical Committee of the University of Leuven. Dietary information was obtained by a semi-quantitative food frequency questionnaire on meat, fish, eggs, milk and cheese. These data reflected only the consumption habits of last year, but they were considered to be indicative for the food consumption during the past years.

2.2. Sample collection and pooling procedure

Approx. 40 ml of blood was collected from each individual. Pooling was done by ranking the women in the order of decreasing daily intake of meat and fish, decreasing daily intake of eggs and

milk, increasing total number of weeks of breast feeding and increasing body mass index. The available serum of three to five subsequently listed individuals was pooled to approx. 50 ml. Each of the 47 pooled samples was divided in two aliquots for the analysis of PCDD/Fs and PCBs (38 ml) and CALUX-TEQ (4 ml).

2.3. Chemical analyses of polychlorinated aromatic hydrocarhons

In 47 pooled serum samples, mono-*ortho* PCBs (PCB 105, 118, 156, 157, 167), indicator PCBs (28, 52, 101, 138, 153, 180) and PCB 44, 66, 74, 99, 110, 128, 149, 170, 183, 187, 194, 199 were measured. These samples were also analysed for the non-*ortho* PCBs (77, 81, 126, 169) and the 17 PCDD/F toxic congeners. The chemical analysis of mono-*ortho*, non-*ortho* PCBs and PCDD/F congeners allowed the calculation of the toxicity equivalents (TEQ) for each sample using the TEF scheme of the WHO (Van den Berg et al., 1998). The chemical analysis methods (low resolution GC–MS for PCBs and high resolution GC–MS for PCDD/F and non-*ortho* PCBs), have been previously described in detail (Koppen et al., 2001).

2.4. CALUX® bioassay

The CALUX® bioassay used in this study was a variant based on a previously described procedure (Murk et al., 1998). In this assay, dioxin-like compounds are assessed via in-vitro activation of the AhR of cultured H4IIE cells (BioDetection Systems BV, Amsterdam, The Netherlands). The method involved *n*-hexane extraction of 4 ml (pooled) blood serum and removal of matrix components by passage through a 33% H_2SO_4 silica column. The extract was partly evaporated and quantitatively transferred to a conical vial for further evaporation. It was reconstituted in dimethyl sulphoxide (DMSO, Acros Organics) for CALUX measurement using rat hepatoma H4IIE cell line transfected with an AhR-controlled luciferase reporter gene construct. Cells were grown in 96-well plates in 100 μl minimal essential medium (α-MEM, Gibco) with 10% feotal calf serum (FCS, Gibco) at a temperature of 37 °C and 5% CO_2. When the cell layer reached 70–80% confluency, the samples and TCDD standards were dosed in quadruplicates to the cells for 24 h. After removal of the medium, cells were washed with 100 μl phosphate-buffered saline without Ca/Mg (PBS-Ca/Mg, Life Technologies) and 30 μl of cell lysis reagent (Promega) was added. The well plates were then shaken for at least 45 mm and stored at −80 °C for at least 1 h. For determination of luciferase activity, the cells were thawed on ice, and 100 μl luciferin assay mix (Promega) was added at room temperature. The light production was measured by a Victor 2 Luminometer (EG and G Wallac). The CALUX-based TEQs were calculated by comparing the luciferase activity induced by the sample against a dose–response curve generated from 2,3,7,8-T_4CDD concentration standards analysed simultaneously. The limit of detection varied with cell growth and volume of the blood samples. This detection limit was calculated as the signal measured from the DMSO solvent control on each well plate plus three times its standard deviation. For 4 ml serum with 700 mg fat/dl the limit of detection was 5.2 ± 3.5 pg TEQ/g fat. Measurements below were set at half of the detection limit. A feotal calf serum sample was run for each series of study samples as internal standard. The inter-experiment variation was about 30% and accepted as normal for this low-loaded sample.

2.5. Statistical analysis

Database management and statistical analysis were performed with Statistica version '99 (Statsoft Inc.). Analytical data that were not normally distributed were log-transformed. Means were compared between the two areas by *t*-test. Pearson correlation coefficients between TEQs obtained by the CALUX-bioassay and GC/MS measured groups of polychlorinated aromatic hydrocarbons were calculated.

3. Results and discussion

3.1. CALUX® bioassay

It was shown (Safe, 1990; Murk et al., 1997) that

that most of toxic actions induced by 'dioxin-like' compounds (such as PCDDs, PCDFs and PCBs) are mediated via the AhR signal transduction pathway. Quantification of the toxic potency of the whole mixture of compounds acting via the AhR pathway would strengthen the causal relationship between observed adverse effect and the presence of 'dioxin-like' compounds as a group. For this purpose, the 'dioxin-like' toxic equivalency factor (TEF) concept was introduced by Safe (1990), allowing conversion of the chemical data set into the AhR-related toxic potency of the mixture of 'dioxin-like' compounds. Concentrations of individual 'dioxin-like' compounds are multiplied by their respective TEF values (Van den Berg et al., 1998) and added together to give the 'dioxin-like' total toxic equivalency (TEQ).

Recently, bioassays (such as CALUX®) have been developed that can measure the total TEQ value of complex mixtures directly, without the need for extensive cleanup and chemical analysis procedures. In the CALUX®-bioassay, TEQs are assessed via in-vitro activation of the AhR of cultured rat hepatoma H4IIE cells. The method involves hexane extraction of the serum sample (4 ml), followed by 33% H_2SO_4 silica cleanup. After exposure of rat hepatoma H4IIE cells with the extract, a light signal of the cell line transfected with an AhR-controlled luciferase reporter gene construct is measured. This signal can be quantified to CALUX-TEQs by use of 2,3,7,8-T_4CDD concentration standards analyzed simultaneously.

TEFs are estimates of relative potency composed of a variety of toxic and biological endpoints. Thus, it cannot be expected that all TEFs are identical or similar to relative potencies (REPs) in a bioassay such as CALUX. Because these values are different, it is not reasonable to expect similar absolute data in extracts from blood analysed by chemical analysis or CALUX®-bioassay.

3.2. TEQ values: chemical analysis versus CALUX®-bioassay

In Table 1, mean concentrations of chemically determined TEQ-values from mono- and non-*ortho* PCBs and PCDD/Fs are given for both regions. Correlation coefficients between chemically measured TEQ values (from PCBs and PCDD/Fs) are presented in Table 2. The mean total TEQ

Table 1
TEQ values for dioxin-like compounds and concentrations of PCBs in 47 pooled samples from 50 to 65 years old women living in two regions of Flanders

	Rural $N_{pool} = 22$	Urban $N_{pool} = 25$	All regions $N_{pool} = 47$
Calux-TEQ (pg TEQ/g fat)			
Calux	37.2 (13.1)	35.0 (16.5)	36.0 (14.9)
TEQ (pg WHO-TEQ/g fat)			
Mono-*ortho* PCB	11.6 (10.7–12.5)	14.2 (13.0–15.6)[b]	12.9 (12.1–13.8)
Non-*ortho* PCB	10.8 (9.3–12.4)	14.5 (12.4–16.9)[b]	12.6 (11.3–14.1)
PCB total	22.5 (20.3–24.9)	29.1 (26.1–32.4)[b]	25.8 (23.8–28.0)
PCDD	24.8 (22.5–27.2)	26.4 (24.0–29.1)	25.6 (24.0–27.4)
PCDF	23.2 (20.2–26.6)	23.1 (20.8–25.7)	23.2 (21.4–25.1)
PCDD+PCDF	47.9 (43.6–52.7)	49.2 (45.0–53.9)	48.6 (45.6–51.8)
Total	70.9 (65.3–76.9)	78.9 (72.7–85.6)	75.0 (70.8–79.5)
PCB (ng/g fat)			
PCB total	498.6 (460.6–539-7)	600.8 (544.0–663.6)[b]	550.6 (514.4–589.3)
Indicator PCB	337.4 (311.8–365.1)	392.0 (355.2–432.7)[a]	365.4 (342.2–390.2)

Mean (SD) or geometric mean (95% confidence interval) are given.
[a] Significantly higher than the other region with $P<0.05$ and $P<0.01$ respectively.
[b] Significantly higher than the other region with $P<0.05$ and $P<0.01$ respectively.

Table 2
Pearson correlation coefficients between the PCB-TEQ, PCDD/F-TEQ and total TEQ measurements, for both regions together (a) and separated per region (b,c; all values were log-transformed

	TEQ	PCB-TEQ	PCDD/F-TEQ	Total TEQ
(a) P+A, $N_{pool} = 47$	PCB-TEQ	–	0.34[a]	0.75[b]
	PCDD/F-TEQ	–	–	0.88[b]
	Total TEQ			–
(b) P, $N_{pool} = 22$	PCB-TEQ	–	0.32	0.64[a]
	PCDDIF/F-TEQ	–	–	0.92[b]
	Total TEQ		–	
(c) A, $N_{pool} = 25$	PCB-TEQ	–	0.39	0.79[b]
	PCDD/F-TEQ		–	0.87[b]
	Total TEQ			–

P = Peer, A = Antwerp. PCB-TEQ = sum TEQ values of non-ortho PCB and mono-ortho PCB, PCDD/F-TEQ = sum, TEQ values from PCDDs and PCDFs, Total TEQ = sum PCB-TEQ and PCDD/F-TEQ.
[a] $P < 0.01$.
[b] $P < 0.001$.

value in pooled serum of 50–65 years old Belgian women was 75 pg WHO-TEQ/g fat. When PCBs were excluded, TEQ values decreased with 34% to 48.6 pg WHO-TEQ/g fat. Similar contribution of PCBs to the total TEQ value (44%) was also observed in Canadian Red Cross blood donors from Toronto (Longnecker et al., 2000). In the latter study, both mean PCDD/F TEQs (20 pg TEQ/g fat) and total TEQs (35 pg TEQ/g fat) were lower than in the present study. Present levels in the studied women group were comparable with values obtained in other industrialized countries about 10 years ago. As in the past, the PCDD/F body burden values in Flanders remain higher than in neighbouring countries, e.g. two times higher than PCDD/F TEQ values measured in a similar German population (43–71 years old) sampled in 1996 (EU, 1999). Also, a study conducted in Wallonia (Belgium) in parallel with the present study showed lower PCDD/F values (mean 36.7 pg I-TEQ/g fat). However, comparison is not completely justified, since the age group was much broader (10–80 years), contained both men and women and was done on individual samples (Bernard, personal communication).

Because of historically elevated values in the Flemish population, it was decided that a more rigorous monitoring programme should be implemented. For this reason, valid methods (in terms of speed, simplicity and accuracy) had to be proposed. One approach could be the use of the CALUX®-bioassay. The total WHO-TEQ values, calculated as the sum of PCDD/Fs, non-*ortho* and mono-*ortho* PCBs TEQ values, were two times higher than the TEQ values determined with the CALUX®-bioassay (36 pg TEQ/g fat). Similar CALUX-TEQ values were observed in 1996–1998 for young Flemish women (mean age 32 years; Pauwels et al., 2000). Considerably higher CALUX-TEQ values (mean of 103.7 pg TEQ/g fat) were measured in 1990–1992 in plasma of Dutch women (mean age 29 years; Brouwer, 1997). However, differences in results might be (partly) explained by differences in fat extraction and efficiency of clean-up methods used. As the CALUX-TEQ reflects synergistic, additive and/or antagonistic interaction of any compound (including 'non-dioxin-like' compounds) with the AhR, sample cleanup is very important.

Bovee et al. (1998) reported similar values for CALUX-assay and TEQs of PCDD/F and several mono- and di-*ortho*-substituted PCBs determined

by GC–MS in 22 cow milk samples. The correlation between the two sets of data was 0.74. The same extraction and cleanup protocol was used for both determinations. In another study, Aarts et al. (1996) have found that average CALUX-TEQ values almost twice as high as the TEQs obtained by GC–MS measurements of PCDD/F, non- and mono-*ortho* PCBs in human milk samples. When they have applied a fat extraction with hexane and fat destruction through a H_2SO_4 silica column, the CALUX-TEQ values were lower or equal to the GC–MS TEQs. In this case, the correlation coefficient between the two sets of TEQ measurements was 0.71. Similar methodology as the latter was used in our study for human serum samples.

When cleanup was not used after fat extraction, Schecter et al. (1999) observed in human blood 1000–4000 times higher CALUX-TEQ values compared to the sum of TEQs from PCDD/F and coplanar PCBs. These higher values suggest that there were other biologically active compounds in human blood samples which could interact and activate the AhR pathway in the CALUX®-bioassay. Possible compounds included dietary compounds such as indole and tryptophan metbolites products, heme breakdown products, as well as polyaromatic hydrocarbons (PAHs), polybrominated biphenyls, polyhalogenated naphthalenes, hexachlorobenzene, azo and azoxybenzenes. In addition, PCBs have been known to act as AhR antagonists in a species-specific manner (Schecter et al., 1999). Depending on the extraction method and cell exposure protocol used, those factors may influence the CALUX-TEQ readings to a higher or lesser extent. Thus, flirther interlaboratory validation and standardization of the CALUX protocol is necessary in order to obtain a valid and comparable tool for biomonitoring.

3.3. Relationships between CALUX-TEQ and total-TEQ

Regardless of the large difference in absolute values, neither for CALUX-TEQs, PCDD/F and total TEQs, there was a regional difference in human serum from elderly women. It was clear that these measurements give similar information concerning relative concentrations of 'dioxin-like' compounds. In Table 3, Pearson correlation coefficients between CALUX-TEQ and other chemically measured WHO-TEQs are given. CALUX-TEQ values were correlated with all other TEQ groups in the range of $r = 0.34–0.57$. The relatively low correlation coefficients indicate that the AhR mediated response from other compounds (probably including non-organochlorines) together with all interaction effects is also measured in the CALUX®-bioassay The correlation coefficient between CALUX measurements and total TEQ value ($r = 0.57$) increased to 0.73 when considering only the women in the urban region (Fig. 1). This important variability in correlation coefficients suggests a difference in the concentration and composition of 'dioxin-like' compounds in human serum. While higher concentrations of most PCB congeners were measured in the women living in the urban area (Table 1), no measurements were done for other compounds with AhR activity. Therefore it was not possible to point to some specific reason for this regional difference in correlation. The observed correlations in the urban area were similar with values found by com-

Table 3
Pearson correlation coefficients between CALUX-TEQ and the different TEQ-measurements for all regions together and separated per region (all log-transformed, except for the CALUX values)

	CALUX®		
TEQs	Rural $N_{pool} = 22$	Urban $N_{pool} = 25$	All regions $N_{pool} = 47$
MO-PCB	0.24	0.61[c]	0.39[b]
NO-PCB	0.36	0.76[c]	0.53[c]
MO+NO-PCB	0.34	0.79[c]	0.51[c]
PCDD	0.52[a]	0.32	0.38[b]
PCDF	0.17	0.50[a]	0.34[b]
PCDD+PCDF	0.39	0.46[a]	0.43[b]
Total TEQ	0.43[a]	0.73[c]	0.57[c]

WHO-TEQ total = sum TEQ values of mono-*ortho* PCB, non-*ortho* PCB, PCDDs and PCDFs. NO-PCB:non-*ortho* PCB, MO-PCB:mono-*ortho* PCB.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

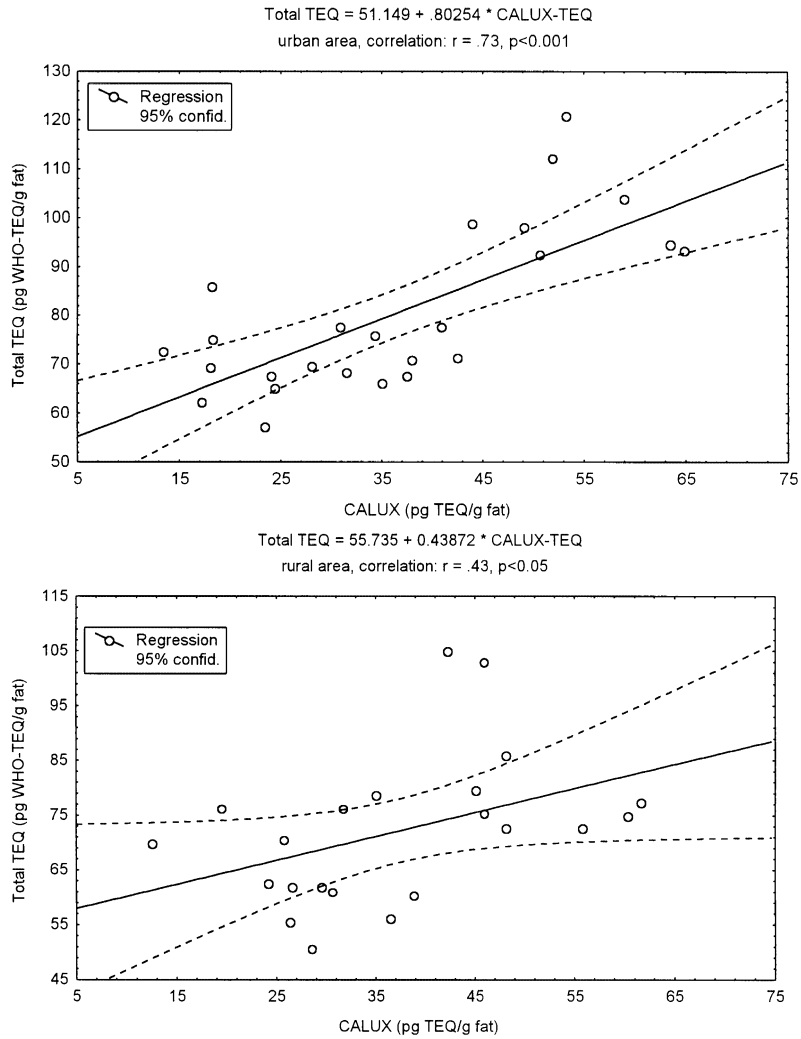

Fig. 1. Correlation between CALUX-TEQ and Total TEQ calculated as the sum of PCDD/F, non-*ortho* PCB and mono-*ortho* PCB WHO-TEQ for 47 pooled serum samples of 50–65 years old women living in two regions of Flanders.

paring GC–MS determined total WHO-TEQs (including PCBs) and CALUX-TEQ in: human serum, $r = 0.71$ (Aarts et al., 1996) and cow's milk, $r = 0.74$ (Bovee et al., 1998).

It was speculated that, for a specific group of specimens, the CALUX®-bioassay could be a possibility for the estimation of the total TEQ without needing large volumes of serum. Thus, for the urban area, using the equation obtained in the regression analysis (Fig. 1), the concentrations of total TEQ could be estimated from the concentrations of CALUX-TEQ. Even knowing that this regression line was valid for exposures to background concentrations as observed for the analysed serum samples of the women, it was still interesting to do this calculation to evaluate its validity for estimations of either the mean or separate sample TEQ-values. The mean estimated total TEQ calculated from observed CALUX-TEQs—using the equation between CALUX-

TEQ and total TEQ — was 75.5 pg TEQ/g fat, being statistically equal to the measured mean of 75 pg total WHO-TEQ/g fat. The difference between observed and estimated concentration of total TEQ was $<20\%$ in 77% of the samples. The maximal discrepancy between the measurements of total-TEQ and the estimated total-TEQ was 40%. This is probably due to the high value for the intercept (51.15 pg TEQ/g fat). This means that whatever a low value for the CALUX-TEQ measurements, the total TEQ (as it would have been chemically measured) is now estimated to be above 50 pg TEQ/g fat in all cases. This is mostly improbable situation because some of the serum samples will have values lower than 50 pg TEQ/g fat (Table 1).

4. Conclusion

It was shown that, especially for comparison of 'dioxin-like' activity between groups of people living in different regions or of different age, CALUX®-bioassay can be a useflil tool for biomonitoring purposes with similar conclusions as deducted from the chemically measured TEQ values. However, a poor correlation between CALUX-TEQs and chemically analysed TEQ values in human serum. The discrepancy in the absolute values needs to be understood in order to use the CALUX®-bioassay as an exposure marker for TEQ values in serum samples is influenced by other unknown factors. Interlaboratory validation and standardization of the protocol will further improve the use of CALUX®-bioassay for biomonitoring purposes.

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References

Aarts, J.M.M.J.G., Cenijn, P.H., Blankvoort, B.M.G., Murk, A.J., Bovee, T.F.H., Traag, W.A., et al., 1996. Application of the chemical-activated luciferase expression (CALUX) bioassay for quantification of dioxin-like compounds in small samples of human milk and blood plasma. Organohalogen Compounds 27, 285–290.

Bovee, T.F.H., Hoogenboom, L.A.P., Hamers, A.R.M., Traag, W.A., Zuidema, T., Aarts, J.M.M.J.G., et al., 1998. Validation and use of the CALUXbioas say for the determination of dioxins and PCBs in bovine milk. Food Addit. Contam. 15, 863–875.

Brouwer A. 1997, Toetsing van het toxicologisch equivalente factoren (TEF) concept voor dioxinen en aanverwante stoffen met behulp van de CALUX-bioassay, Rapport CALUX-project.

Covaci, A., Koppen, G., Van Cleuvenbergen, R., Schepens, P., Winneke, G., Van Larebeke, N., et al., submitted for publication1. Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 2: Correlations among PCBs, PCDD/PCDFs and the use of predictive markers. Chemosphere.

EU. 1999, Dioxin Exposure and Health Data, Report produced for European Commission Environment, UK. http://europa. eu.int/comm/environment/dioxin.

Koppen, G., Covaci, A., Van Cleuvenbergen, R., Schepens, P., Winneke, G., Nelen, V., et al., submitted for publication1. Persistent organochlorine pollutants in human serum of 50-65 years old women in the Flanders Environmental and Health Study (FLEH S). Part 1: Concentrations and regional differences. Chemosphere.

Longnecker, M.P., Ryan, J.J., Gladen, B.C., Schecter, A.J., 2000. Correlations among human plasma levels of dioxin-like compounds and polychlorinated biphenyls (PCBs) and implications for epidemiologic studies. Arch. Environ. Hlth. 55, 195–200.

Murk, A.J., Leonards, P.E.G., Bulder, A.S., Jonas, A.S., Rozemeijer, M.J.C., Denison, M.S., et al., 1997. The CALUX assay adapted and validated for measuring TCDD equivalents in blood plasma. Environ. Toxicol. Chem. 16, 1583–1589.

Murk, A.J., Leonards, P.E.G., van Hattum, B., Luit, R., van der Weiden, M.E.J., Smit, M., 1998. Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environ. Toxicol. Pharmacol. 6, 91–102.

Pauwels, A., Cenijn, P.H., Schepens, P.J.C., Brouwer, A., 2000. Comparison of chemical-activated luciferase gene expression bioassay and gas chromatography for PCB determination in human serum and follicular fluid. Environ. Hlth. Perspect. 108, 553–557.

Safe, S., 1990. Determination of 2,3,7,8-TCDD toxic equivalent factors (TEFs), support for the use of the in vitro ALIII induction assay. Chemosphere 28, 791–802.

Schecter, A.J., Sheu, S.U., Bimbaum, L.S., De Vito, M.J., Denisson, M.S., Päpke, O., 1999. A comparison and discussion of two different methods of measuring dioxin-like compounds: gas chromatography-mass spectrometry and the CALUX bioassay—implications for health studies. Organochlorine Compounds 40, 247–250.

Tarkowski, S., Yrjänheikki, E., 1989. WHO-coordinated intercountry study on levels of PCDDs and PCDFs in human milk. Chemosphere 19, 553–557.

Van den Berg, M., Bimbaum, L.S., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., et al., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife. Environ. Hlth. Perspect. 106, 775–792.